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Toxic Limits of Drugs and other Chemical Substances

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TOXIC LIMITS OF DRUGS AND OTHER CHEMICAL SUBSTANCES

BY

EDWARD ADELBERT DOISY

A.B. University of Illinois, 1914

THESIS

Submitted in Partial Fulfillment of the Requirements for the

Degree of

MASTER OF SCIENCE

IN CHEMISTRY

IN

THE GRADUATE SCHOOL

OF THE

UNIVERSITY OF ILLINOIS

1915

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May 31, 1915.

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Edward Adelbert Doisy,

ENTITLED Toxic Limits of Drugs and Other Chemical Substances.

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science in Chemistry

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Recommendation concurred in:*

Committee on Final Examination*

*Required for doctor's degree but not for master's.

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TOXIC LIMITS OF DRUGS AND OTHER CHEMICAL SUBSTANCES.

I INTRODUCTION.

1. Statement of problem.

This research was undertaken with two definite purposes in view. (1) To establish a method which would be universally applicable in determining the toxicity of any soluble substance. (2) After the best method possible, upon a careful consideration of the results of other investigators and our own experimental data, has been decided upon, to use it in determining the toxicity of a number of compounds which are poisonous to man. The research then resolves itself into establishing a biological method for determining toxicity and the determination of the minimum lethal dose of a number of common substances by this method.

2. Necessity of a biological method.

Altho several pharmacologists and toxicologists have attempted to define a poison, no satisfactory definition has ever been given. In medico-legal cases it would be very important if we were able to say, "This is a poison; that is not." Blyth states that "a substance may be called a poison if it is capable of being taken into the living organism and causes by it's own inherent chemical nature impairment or destruction of function." This is a very good definition but it fails in the one respect, that it is too comprehensive for practical purposes. Medicines are administered to change the conditions existing in some particular organ or tissue. Under Blyth's formula, they would be poisons. Again, sodium chloride, if ingested in very large quantities will cause death; but who would ever think of classing it as a poison? It is normally present in all tissues in

small amount and is essential to the body for proper nutrition. If Blyth's definition were interpreted literally even water would become a poison. Copious water drinking will reduce slightly the specific gravity of blood and more markedly the density of urine.

After careful consideration, it seems that Blyth's definition would be satisfactory if a statement regarding the size of the dose were added to it. Toxicologically, it would be improper to call many compounds poisonous which would be classed as such by physiologists. Since there is this difference in view-point between physiologists and toxicologists, some way must be devised for use in medico-legal cases by which it can be definitely stated that a certain dose of a given substance is a poison.

In addition to its use in medico-legal cases, an accurate knowledge of toxicity would prevent many cases of accidental poisoning. At present there is a woeful lack of knowledge regarding poisons. The public can not be expected to handle compounds carefully if they do not know of their toxic qualities. When poisons are sold indiscriminately and with only slight, if any, warning, accidental poisoning is certain to occur.

This lack of knowledge regarding poisons is suffered by others as well as the general public. Physicians and pharmacists should know the exact toxicity of the substances that they sell. If they realized how toxic some supposedly safe compounds are, they would handle and sell them with greater caution. If there were a table of toxicities, physicians could easily determine what dose it would be unsafe to exceed. To simplify matters for the dealer and to insure the safety of the public, a table of toxic coefficients should be worked out and all substances sold with reference to it. If a substance is a powerful poison, sell it only under very strict

conditions; if it is only moderately poisonous, then sell it under less strict conditions. In the former case the label should be very noticeable and should have on it the number of toxic units per cubic centimeter or per gram.

In the preceding paragraphs, it has been shown that a definition of a poison is very desirable. It will be apparent upon a consideration of the subject that the ideal way would be to define a poison on its toxicity toward man. This seems to be impossible, as cases of accidental poisoning or suicide are the only sources of data. It is rarely possible to determine the size of the dose taken in these cases. Even then, we do not know how much less would have been fatal. Since a poison can not be defined in terms of its toxicity toward man, some other means of definition must be found. This suggests the use of a test animal.

II. METHODS THAT HAVE BEEN USED IN DETERMINING THE ACTIVITY OF DRUGS.

1. Early work in biological standardization.

As early as 1865 Fagge and Stevenson[2] claimed that physiological tests would become very important in medico-legal cases. As chemical methods were not available, they standardized digitalis biologically using the frog as a test animal. Their method was to attach a weighed frog to a piece of cork and to expose the heart, a definite dose was injected and the time for systolic stand-still determined. They chose the frog in preference to higher animals because it showed no fear and the drugs acted quickly. They found that seasonal variations did not occur.

No other work of note in biological standardization was done until Koppe[3], in 1875, showed that the resistivity of frogs of different species toward digitalis is not the same. Rana temporaria

were found to be more susceptible than Rana esculenta. Cats, rabbits and dogs were also used by this investigator in determining the toxicity of digitalis. A few other investigators used animals in testing drugs, but the work rather drifted along and no practical application was pointed out until 1898.

In the early years of the decade 1890 - 1900, the work on production and testing of antitoxin was started and pushed along quite rapidly. The difficulty of standardizing antitoxins was encountered in the beginning and a biological method appeared to be the only one available. This idea was seized upon and under the impetus given it by Ehrlich, Behring and others the methods were rapidly developed and put into use. When this work came to the attention of Houghton, he conceived the idea that digitalis and other drugs that can not be standardized chemically, might be tested biologically.

2. Methods of Digitalis Standardization.

(a) Twelve hour (Houghton[4]) frog method.

In his preliminary work Houghton tried out rats, rabbits, guinea-pigs and frogs as test animals. From the results which he obtained he concluded that frogs were the most suitable. The technique which he used is as follows: The sample of digitalis to be tested is dissolved in normal saline solution, the total volume of the dose should not exceed 0.5 cc. Injection is made with a sharp-pointed glass pipette through the frog's mouth into the abdominal lymph sac. The end-point was taken as the smallest amount which causes death within twelve hours. By varying the size of the dose and by running several series the approximate lethal dose can be determined. He found that it was necessary to maintain the same

conditions thruout his experiments and that even slight changes affected his results. It seemed advisable to keep a standard solution on hand and first standardize the frogs, as it were, then standardize the unknown. Houghton used frogs because of their cheapness, ease with which they are cared for, and accuracy of results. The chief objection to this method as given out is that the doses were not given in proportion to the weight of the animal.

(b) The cat method (Hatcher's[5]).

The cat is anaesthetized with morphine and ether and tied to an animal board. Then a dissection of the femoral vein is made, into which a cannula is introduced. The digitalis solution, properly diluted, is run in from a burette at such a rate that death shall occur in about ninety minutes. The cat unit is taken as the smallest amount of crystalline ouabain that will cause death within ninety minutes. The activity of other members of the digitalis series is calculated on this as a standard. Hatcher used cats in his experiments because of their cheapness, the facility with which they may be obtained and handled. His results with this method were very accurate.

Of several investigators which have considered this method only one has reported favorably on it. Eggleston[16] says that it is the best method for standardizing digitalis because: - 1. It is accurate to within 10%. 2. It gives constant results from year to year. 3. It tests the action of the drug upon which its therapeutic use depends. 4. It is short and inexpensive. 5. Widely different preparations can be accurately compared. 6. Results are transferable to man.

On the other hand, neither Hale[6] nor Haskell[7] favor this

method for digitalis assay. Both agree that the technique is quite difficult and that only a skillful pharmacologist can get satisfactory results. Hale states that it is difficult to secure cats in sufficient numbers; and that cats are apt to become sick if kept confined. Eckler[8] says that he has gotten variations in the toxic dose of as much as 123%. Since first reporting on this method Hatcher[5] has made some more assays. In a few cases he noted a variation of as much as 50%.

(c) Guinea-pig method (Reed and Vanderkleed[9]).

The technique of an important method which uses the guinea-pig as a test animal is as follows: The animals are weighed and doses in proportion to their weight are administered. The minimum lethal dose is determined in this way and then administered to five or six other guinea-pigs as controls. The end-point is taken as death within two hours. Githens and Vanderkleed[10] in discussing the use of this animal state that its resistance shows no variation because guinea-pigs are always of the same species. They are easily obtained; are large enough to render easy the measurement and calculation of the dose; and are very resistant to alcohol. This latter fact is particularly valuable in standardizing fluidextracts and tinctures.

Although Githens and Vanderkleed[10] report favorably on this method, Hale[6] has raised two or three objections to the use of the guinea-pig. He reports that the cost of an assay would be about $5.00. Therefore, it would be too expensive to be used as a routine method. Another important factor is its lack of accuracy. In some of Hale's assays, guinea-pigs often survived 20% larger doses than the fatal dose for other guinea-pigs.

Walters and Haskell[11] reported that the individual variation to ouabain might be as much as 25%. They also found that variations in resistance depend upon age, weight, season, source of animals, and food. A diet of oats with a minimum of green food seems to cause a decided increase in the susceptibility of guinea-pigs; a diet of green foods exclusively seems to decrease the susceptibility.

(d) Gold-fish method.

Quite an innovation has been introduced by Vanderkleed and Pittenger[12] (April, 1915) in digitalis standardization in that they employed gold-fish as test animals. The fish were put directly into digitalis solutions of varying concentrations. Since there is a large surface exposed and the walls are quite thin, the drug is taken into the circulation very rapidly thru the gills. The results of the experiments performed indicate that accurate results can be obtained by this method. It is probable that temperature is the only outside factor which influences the resistivity of the fish. Too much dependence must not be placed upon this method, since only a preliminary series of experiments have been finished by Vanderkleed and Pittenger, and no other investigators have yet tried it out.

3. Phenoloid standardization.

In 1913, Hale's[13] work on the standardization of the phenoloid disinfectants appeared. It was carried on because of the large number of cases of accidental poisoning due to the improper use and careless labeling of this class of substances. Hale developed the following technique for determining the phenol coefficient of a solution: Injections were made subcutaneously on the white

mouse. The volume of the dose, which was given in proportion to the weight of the animal, lay within the limits 0.03 - 0.04 cc. per gram body weight. Since the results of a large number of experiments showed that a mouse either died or recovered in twenty-four hours, and since this interval was very convenient for carrying on the assays, it was adopted as the time limit. Hale seems to indicate that weight has an effect on the minimum lethal dose, as he specifies that the mice shall be from 15 - 30 gms. weight. In his standardizations phenol is taken as a standard. In every case of toxic coefficient determination the minimum lethal dose of phenol and of the unknown are determined at the same time. Apparently, he realizes that certain factors affect the resistance of the white mouse.

4. General summary of methods and discussion of test animals.

(a) Frog.

Several points in favor of the use of the frog as a test animal might be mentioned. The expense would be very small, since only about two dozen animals would be necessary for an assay. The frogs which are used are too small to be eaten and therefore can be purchased at a low figure (25 - 35 ¢ per dozen). The cost of a standardization then would be less than $1.00. Frogs are easily injected and the technique is very simple.

Objections that could be offered to the frog as a test animal are rather numerous. Most investigators have found that frogs show wide variations in resistance which depend upon sex, age, weight, season, species and temperature. A change in any one of these factors causes appreciable errors. Frogs are kept in a healthy, normal condition with more difficulty than animals with whose characteristics and habits of life we are more familiar. Another

fundamental objection which arises is the fact that the frog is a cold-blooded animal. Consequently, certain anatomical and physiological differences between this animal and mammals occur. Because of these differences, peculiarities in the reaction toward particular drugs are apt to be found. For these reasons it seems probable that results which are obtained on frogs would be less valuable than those on an animal which is more closely related to man.

This might suggest the use of dogs or cats. While either animal probably would give very good results, certain facts render their use undesirable. First, we must consider the expense of procuring them; then the expense and difficulty of keeping them. When dogs are confined in strange quarters they usually howl and whine. Some mis-directed person notices it and reports it to the anti-vivisectionists who make trouble for the scientist. The main objection, however, to the use of cats or dogs is that they can not be procured in sufficient numbers for the assays which are to be made. Even if they could, their cost would render the expense of an assay prohibitive.

Rabbits have been considered as a possible test animal, but rejected for several fundamental reasons. They show more individual variations and idiosyncrasies than other animals. It is also a generally accepted fact that their resistivity to unfavorable conditions is not great. This makes it necessary to handle them with the utmost caution. Even then, results probably would not check up well because of the extreme susceptibility of rabbits.

An animal whose use has been advocated strongly by some investigators is the guinea-pig. In the testing of antitoxin suc-

cessful results have been obtained, but after a consideration of the work of Hale[6], and Walters and Haskell[11] it was seen that this animal would not be suitable for toxicological assay. Apparently, it has been pretty well proven that the resistivity of the guinea-pig changes with variations in sex, age, weight, temperature, food and season. Therefore, to get accurate results with any method which employs the guinea-pig as a test animal it would be necessary to use some substance as a standard, and to run a series of controls with each assay. This would almost double the cost of a standardization, which cost Hale found amounted to approximately $5.00. The fact that the guinea-pig is a vegetarian must not be forgotten. This matter of food would probably cause peculiarities in the reaction toward some drugs, which would render the results non-transferable to man. This would in a certain measure defeat the purpose of the research.

We will now consider an animal which does not have this disadvantage of being a vegetarian, and the one which appears to us to be the most free from objectionable features. The white mouse has been used by a few investigators in toxicity determinations. Reid Hunt[14] used it in the examination of the effect of feeding thyroid. He says that it is necessary to use animals of approximately the same age and weight, which have been kept under the same conditions upon the same kind of food. At Frankfort, Germany, he found that the minimum lethal dose of acetonitrile was 0.7 mgm. per gram body weight; at Washington, D. C., 0.15 mgm. While it is probably true that the resistance of the white mouse varies with age, sex, temperature, food and weight, we consider it to be the best animal for the determination of toxic units. It is small,

easily cared for, will thrive upon almost any kind of food and will remain healthy if properly cared for. Mice are cheap, costing about 10 ¢ – 15 ¢ each. If the investigator so desires he may grow his own stock very readily. White mice are rapid breeders, multiply quickly and require but little care in breeding. They eat but little and their food is inexpensive. They stand close to man in the animal scale and grow nicely upon the food that man eats. From this we would expect that the number of peculiarities in reaction toward any drugs would be few. Of course, the more directly transferable to man a table of toxic coefficients is, the greater its value. The mouse is a small animal and has a gentle nature. One man can handle them and make the injections quite easily. For these reasons we decided that the white mouse would be the most suitable animal for the determination of toxic units.

III. METHOD OF STANDARDIZATION USING THE WHITE MOUSE.

After deciding to use the white mouse it was necessary to develop a technique and to determine the exact conditions to be used. Series of experiments were carried on to determine what factors influence the susceptibility of the white mouse. It was decided to use caffeine and morphine sulphate in these determinations. One condition in the experiment was altered while the others were kept constant. If this alteration had no effect, the dose which was just lethal would not be changed.

1. Influence of various factors.

Effect of sex – Caffeine.

Sex	Weight	Dose per gm.	Result
Male	17.5 gm.	0.0003087 gm.	survived
"	12.7 "	0.0003166 "	"
"	15.2 "	0.0003224 "	died.

Sex	Weight	Dose per gm.	Result
Female	13.6 gm.	0.0002402 gm.	survived
"	11.7 "	0.0002685 "	died
"	14.2 "	0.0002964 "	"
"	15.8 "	0.0003101 "	"

Effect of sex - Morphine sulphate

Sex	Weight	Dose per gm.	Result
Male	24.3 gm.	0.0006671 gm.	survived
"	22.6 "	0.0006844 "	"
"	23.3 "	0.0006958 "	died
"	21.6 "	0.0007247 "	"
Female	27.4 "	0.0006121 "	survived
"	26.4 "	0.0006494 "	died
"	20.6 "	0.0006694 "	"
"	21.5 "	0.0007194 "	"

Effect of age - Caffeine. Males used.

Age	Weight	Dose per gm.	Result
Adult	23.0 gm.	0.0003209 gm.	survived
"	25.0 "	0.0003267 "	"
"	20.0 "	0.0003298 "	died
"	24.5 "	0.0003334 "	"
3 wks.	6.4 "	0.0002160 "	survived
"	5.8 "	0.0002383 "	died
"	5.2 "	0.0002658 "	"
"	5.2 "	0.0002900 "	"

Effect of age - Morphine sulphate. Males used.

Age	Weight	Dose per gm.	Result
Adult	24.3 gm.	0.0006671 gm.	survived
"	22.6 "	0.0006844 "	"
"	23.3 "	0.0006958 "	died
"	21.6 "	0.0007247 "	"
3 wks.	4.6 "	0.0004162 "	survived
"	6.8 "	0.0004317 "	died
"	5.9 "	0.0004327 "	"

The mice upon which our assays were made were fed upon a mixed feed consisting of sunflower seed, cracked corn, wheat, dog biscuit and bologna. To determine whether the kind of food affects the susceptibility of the white mouse, some mice were fed upon a special diet consisting of sunflower seed, cracked corn and wheat for a period of about three weeks before making the injections.

Effect of food - Caffeine. Males used.

Food	Weight	Dose per gm.	Result
Normal	23.0 gm.	0.0003209 gm.	survived
"	25.0 "	0.0003267 "	"
"	20.0 "	0.0003298 "	died
"	24.5 "	0.0003334 "	"
Special	19.4 "	0.0002491 "	"
"	17.4 "	0.0002763 "	"
"	19.4 "	0.0002999 "	"

Effect of food - Caffeine. Females used.

Food	Weight	Dose per gm.	Result
Normal	22.9 gm.	0.0002414 gm.	survived
"	28.2 "	0.0002601 "	"

Food	Weight	Dose per gm.	Result
Normal	23.6 gm.	0.0002725 gm.	died
"	22.9 "	0.0002809 "	"
Special	16.6 "	0.0001695 "	survived
"	15.2 "	0.0001851 "	"
"	14.8 "	0.0002037 "	died
"	14.2	0.0002265 "	"

Effect of food - Morphine sulphate. Males used.

Food	Weight	Dose per gm.	Result
Normal	24.3 gm.	0.0006671 gm.	survived
"	22.6 "	0.0006344 "	"
"	23.3 "	0.0006958 "	died
"	21.6 "	0.0007247 "	"
Special	21.6 "	0.0003015 "	survived
"	15.4 "	0.0003483 "	died
"	11.4 "	0.0004033 "	"
"	15.7 "	0.0005246 "	"
"	19.6 "	0.0006059 "	"

Effect of temperature - Caffeine. Males used.

Temp.	Weight	Dose per gm.	Result
30°	31.2 gm.	0.0002673 gm.	survived
"	28.2 "	0.0002851 "	died
"	30.8 "	0.0002937 "	"
20°	19.3 "	0.0002604 "	survived
"	20.1 "	0.0002700 "	died
"	26.6 "	0.0002796 "	"
10°	31.0 "	0.0001037 "	survived

Temp.	Weight	Dose per gm.	Result
10°	20.4 gm.	0.0001478 gm.	died
"	24.5 "	0.0001805 "	"
"	21.3 "	0.0002213 "	"

From the results of the series of experiments carried on it may be seen that the food, age and weight, sex and temperature affect the susceptibility of the white mouse toward caffeine and morphine sulphate. It is almost certain that this would hold true for any other substances. Reasoning from this we adopted the following set of conditions under which our toxicological assays were made:

2. Method used in biological standardization.

Adult male white mice of from 15 - 30 grams weight were used. They were regularly fed upon a mixed diet of sunflower seed (2 parts), wheat (1 part), and cracked corn (1 part) and were given a piece of dog biscuit often enough so that there was a piece in each cage at all times. Twice a week they were given a small piece of bologna. Mice which are received from dealers should be kept upon this food for at least ten days before use in making assays.

This latter requirement could be dispensed with and white mice from any source and kept under any uniform conditions could be used if some substance were adopted as a standard and other substances standardized on it as a base. An assay of the standard and of the urknown should be carried on at the same time and under the same conditions. If this procedure were followed and enough animals used to rule out individual variation, results accurate to within 5%, probably, could be obtained.

The technique of injection as carried out in our experiments

is quite simple.. One cubic centimeter, all-glass hypodermic syringes graduated in 1/100 were used. The syringe was kept in alcohol between injections. The volume of the dose was made to come within the limits .03 - .04 cc. per gram. Distilled water was used as a solvent. The mouse was accurately weighed to the first decimal and the dose calculated accordingly. A glove was worn on the left hand. The left fore-paw was grasped between the thumb and forefinger and the tail held between the third and fourth fingers. Injection was made with the right hand, the puncture being made near the tail, on the back, on either the left or right side of the spinal column. After injection the mouse was put into a cage in a room where the temperature was constant and given food and water. The dose which just caused death within twenty-four hours was taken as the minimum lethal dose.

IV. DATA AND DISCUSSION.

1. Determination of minimum lethal dose under stated conditions.

Using method described, the minimum lethal dose of several substances was determined as accurately as possible.

Ethyl alcohol. (second lot)

Weight	Dose per gm.	Result
25.4 gm.	0.007361 gm.	survived
24.8 "	0.007742 "	"
23.1 "	0.008094 "	died
26.9 "	0.008171 "	"
27.6 "	0.008513 "	"

Apomorphine hydrochloride, (second lot)

Weight	Dose per gm.	Result
22.7 gm.	0.0001481 gm.	survived

Weight	Dose per gm.	Result
20.7 gm.	0.0001498 gm.	survived
21.2 "	0.0001605 "	died
23.3 "	0.0001703 "	"

Caffeine . (second lot)

Weight	Dose per gm.	Result
21.4 gm.	0.0003360 gm.	survived
15.8 "	0.0003558 "	"
21.4 "	0.0003666 "	died
18.4 "	0.0003695 "	"

Chloral hydrate. (third lot)

Weight	Dose per gm.	Result
22.0 gm.	0.0008000 gm.	survived
21.3 "	0.0008077 "	"
23.3 "	0.0008148 "	died
18.9 "	0.0008266 "	"

Cocaine hydrochloride. (second lot)

Weight	Dose per gm.	Result
22.4 gm.	0.0001504 gm.	survived
22.3 "	0.0001609 "	"
17.7 "	0.0001658 "	died
11.4 "	0.0001716 "	"

Hyoscine hydrobromide. (third lot)

Weight	Dose per gm.	Result
28.2 gm.	0.0008634 gm.	died
25.3 "	0.0008730 "	survived
26.5 "	0.0008903 "	"
22.4 "	0.0008919 "	died

Weight	Dose per gm.	Result
17.2 gm.	0.0009004 gm.	died
20.2 "	0.0009071 "	survived

Morphine sulphate. (second lot)

Weight	Dose per gm.	Result
24.3 gm.	0.0006671 gm.	survived
22.6 "	0.0006844 "	"
23.3 "	0.0006958 "	died
21.6 "	0.0007247 "	"

Potassium nitrate. (third lot)

Weight	Dose per gm.	Result
22.2 gm.	0.0019970 gm.	survived
29.7 "	0.0021090 "	"
23.9 "	0.0021443 "	died
24.8 "	0.0021940 "	"

Sodium benzoate. (third lot)

Weight	Dose per gm.	Result
30.4 gm.	0.001211 gm.	survived
25.0 "	0.001236 "	"
22.1 "	0.001263 "	died
17.8 "	0.001295 "	"
18.6 "	0.001311 "	"

Sodium cinnamate. (third lot)

Weight	Dose per gm.	Result
25.6 gm.	0.001167 gm.	survived
20.7 "	0.001194 "	"
30.3 "	0.001205 "	"
19.4 "	0.001221 "	died
36.3 "	0.001254 "	"

Weight	Dose per gm.	Result
20.2 gm.	0.001337 gm.	died

Caffeine. (third lot)

Weight	Dose per gm.	Result
31.2 gm.	0.0002673 gm.	survived
28.2 "	0.0002851 "	died
30.8 "	0.0002937 "	"

Morphine sulphate. (third lot)

Weight	Dose per gm.	Result
23.6 gm.	0.0005763 gm.	survived
25.8 "	0.0005865 "	died
25.5 "	0.0006085 "	"

2. Choice of a standard.

The toxicities of the compounds mentioned in the foregoing tables have been accurately determined. It will have been noticed that two values were given for both morphine sulphate and caffeine. In this work two shipments of mice were received from a dealer. In order to make our results strictly comparable both lots were used in determining the minimum lethal dose of morphine sulphate and caffeine. Results obtained with the former alkaloid were not regular; peculiar reactions sometimes occurred; and its minimum lethal dose toward the white mouse and toward man are not closely related. The test animal, if a comparison is made with the toxicity of other alkaloids, evidently has a greater resistance than man possesses toward it. (These peculiarities might be explained by the fact that the purity of the sample can not be vouched for). On the other hand, however, good consistent results have been obtained with caffeine. Its toxicity toward the white mouse and to-

ward man seem to be rather closely related. Therefore, we have provisionally adopted it as a standard in order to correlate our data and to get an idea of relative toxicity.

This variation in resistivity of mice of different shipments suggested the idea that some chemical should be adopted as a standard and other substances standardized on it as a base. Parallel assays under identically similar conditions should be run upon the unknown and standard. In this way only could accurate results with biological standardization be obtained. However, if mice are grown and experimented with under exactly the same conditions close approximations to the true results can be secured. Hale working in the Hygienic Laboratory at Washington found the minimum lethal dose for phenol to be 0.00045 gram per gram body weight. Upon mice grown in our laboratory we found the toxic dose to lie between 0.00041 and 0.00046 gram. This data is given to show that results, using the white mouse as a test animal, can be duplicated with a fair degree of accuracy by different investigators in different parts of the country. We believe, in spite of this, that it would be much wiser and that better results could be obtained by the use of a standard, as has already been suggested.

3. Determination of approximate lethal dose.

In addition to the data on accurate assays which has been given, the approximate lethal dose of several other substances has been determined. These determinations were not carried on under exactly standard conditions but the results are sufficiently accurate to give an idea of the toxic values of the following compounds:

Substance	Dose per gm. body weight	
	survived	died
Aconitine	0.000000306 gm.	0.000000425 gm.
Ammonium benzoate	0.001364 "	0.001409 "
Atropin hydrochloride . . .	0.0003898 "	0.000459 "
Barium chloride($BaCl_2.5H_2O$)	0.0000696 "	0.0000958 "
Codeine sulphate	0.0001593 "	0.0002040 "
Coniine	0.00006796 "	0.00006837 "
Glycerin	0.013597 "	0.013986 "
Hydrastine hydrochloride ..	0.000222 "	0.000247 "
Lead acetate		
$[Pb(C_2H_3O_2)_2.3H_2O]$. .	0.007925 "	0.008235 "
Nicotine	0.00001984 "	0.00002362 "
Oxalic acid	0.000222 "	0.000267 "
Phenol	0.0004154 "	0.0004623 "
Potassium benzoate	0.001714 "	0.001800 "
Potassium chlorate	0.001003 "	0.001010 "
Quinine hydrochloride . . .	0.0003585 "	0.0003640 "
Sodium salicylate	0.000960 "	0.001004 "
Sodium sulphate	0.005002 "	0.005516 "
Sparteine sulphate	0.0000738 "	0.0000796 "
Strychnine sulphate	0.00000129 "	0.000001357 "
Theophylline	0.0002272 "	0.0002380 "
Veronal	0.0006511 "	0.0007027 "

4. Toxic coefficients with caffeine as a standard.

The results of the assays as given in this and in other tables may be made more apparent and at the same time put in a more convenient form by preparing a table of toxic coefficients.

Arbitrarily taking the toxicity of caffeine as 100, the toxicities of the other compounds studied were calculated according to the formula

$$\frac{\text{Minimum lethal dose of caffeine}}{\text{Minimum lethal dose of the substance}} = \frac{\text{toxicity of the substance}}{100}$$

Substance	Toxic coefficient
Aconitine	76988
Strychnine sulphate	24112
Nicotine	1385
Coniine	478
Sparteine sulphate	411
Barium chloride ($BaCl_2.5H_2O$)	342
Apomorphine hydrochloride	228
Cocaine hydrochloride	221
Codeine sulphate	160
Theophylline	137
Hydrastine hydrochloride	132
Oxalic acid	123
Caffeine	100
Quinine hydrochloride	90
Atropine hydrochloride	71
Phenol	71
Morphine sulphate	53
Veronal	47
Alcohol	45
Chloral hydrate	35
Sodium salicylate	33
Potassium chlorate	32
Hyoscine hydrobromide	32

Substance	Toxic coefficient
Ammonium benzoate	23
Sodium benzoate	23
Sodium cinnamate	23
Potassium benzoate	18
Potassium nitrate	13.3
Sodium sulphate	6
Lead acetate	4
Glycerin	2

5. Attributes of a good standard.

This table which uses caffeine as a base shows exactly what is desired - the great toxicity of aconitine and strychnine and the low toxicity of certain inorganic salts such as Na_2SO_4 and KNO_3. It seems to the writer that a good standard must possess several other qualities. It must have an average toxicity because a substance with either a high or low minimum lethal dose would give less accurate results. The reaction would be less definite and the amount administered could not be measured as accurately in either case. The standard should exert practically no local action, death being due entirely to the action of the substance after its absorption from the point of injection. It is necessary that the substance be sufficiently soluble in distilled water so that its minimum lethal dose may be determined without injecting an undue amount of solvent. It would be wiser to choose for a standard something with which the public is not familiar. This is of little scientific interest, but for practical purposes it is desirable. For instance, if quinine should be chosen and we stated that some chemical has a toxicity ten times as great, most

people would not consider it particularly dangerous. It would in reality be a strong poison, yet a statement of its property in this way would not insure the necessary precautions in handling it. People are so accustomed to the taking of quinine that they do not regard it as a poison. A substance of this kind would not serve satisfactorily as a standard.

6. Caffeine as a standard.

Caffeine, too, is more or less well known to the public from its association with coffee. They know of it thru the constant agitation which points out the harmful effects of caffeine in coffee. It might be said that stating toxicity in terms of caffeine would cause an exaggerated care in the use of substances which are almost innocuous. We hardly believe that this would be true except in a few cases, because it is human nature to become careless. Another thing to be considered is that human life is valuable and that we can not be too careful in preserving it. If men are willing to work during the best years of their lives in discovering methods of treating the sick and caring for the well, we can certainly well afford to use care in handling poisons. In one other and perhaps the most important respect, caffeine fulfills the requirements of a good standard. In our experiments no individual variations or idiosyncrasies toward it were noted. If the assays are carried out from beginning to end under the same conditions, results can be duplicated. Therefore, we can say that caffeine meets the most insistent demand of a good standard. One other point in its favor is that it can be secured in a pure condition. This is essential for a standard because if its composition varied with different samples good results could not be hoped for.

7. Potassium nitrate as a standard.

Altho it seems to the writer that caffeine is the best substance studied for use as a standard, potassium nitrate might be used with almost as good results. Its injection into mice produces sharp, definite reactions and it is an inorganic substance which may be obtained in a pure state. A table calculated on it as a base with a toxicity of 10 would be similar to the table of toxic coefficients calculated upon caffeine as a standard. Two points might be brot up against its use. Altho its chief action occurs after its absorption, it exerts a slight local action. The other objection is that people are liable to relate its toxicity to that of common salt or to that of saltpeter with which they are familiar in meat preservation, and thereby lose respect for a table which uses it as a standard. For comparison a table which was calculated using KNO_3 as a base with a toxicity of 10 has been prepared.

Substance	Toxic coefficient
Aconitine	57886
Strychnine sulphate	13126
Nicotine	1041
Coniine	359
Sparteine sulphate	309
Barium chloride ($BaCl_2.5H_2O$)	257
Apomorphine hydrochloride	171
Cocaine hydrochloride	166
Codeine sulphate	120
Theophylline	103
Hydrastine hydrochloride	99
Oxalic acid	92
Caffeine	75

Substance	Toxic coefficient
Quinine hydrochloride	68
Atropine hydrochloride	53
Phenol	53
Veronal	35
Alcohol	34
Chloral hydrate	26
Sodium salicylate	25
Hyoscine hydrobromide	24
Potassium chlorate	24
Sodium benzoate	17
Sodium cinnamate	17
Ammonium benzoate	17
Potassium benzoate	14
Potassium nitrate	10
Sodium sulphate	4.5
Lead acetate $Pb(C_2H_3O_2)_2 \cdot 3H\ O$	3
Glycerin	1.5

8. Toxicity toward man.

It was thot that a table which shows the toxicity of the compounds studied toward man would be a valuable addition to this paper. Altho the toxic dose for human beings as given in books on toxicology is only roughly approximate, average values can be taken and a table prepared which will throw some light on the value of the white mouse in toxicological assay.

Substance	Fatal dose for adult.	Fatal dose for adult man per gm. body weight.	Fatal dose for white mouse per gram body weight.
Aconitine	0.003 gm.	0.000000043 gm.	0.00000036 gm.
Strychnine	0.045 "	0.00000064 "	0.00000132 "

Substance	Fatal dose for adult.	Fatal dose for adult man per gm. body weight.	Fatal dose for white mouse per gram body weight.
Nicotine	0.065 gm.	0.00000093 gm.	0.000022 gm.
Coniine	0.130 "	0.00000186 "	0.000068 "
Atropine	0.225 "	0.0000032 "	0.000042 "
Morphine	0.300 "	0.0000043 "	0.00069 "
Codeine	0.520 "	0.0000074 "	0.00018 "
Cocaine	1.00 "	0.000014 "	0.00016 "
Phenol	3.9 "	0.000056 "	0.00045 "
Oxalic acid	3.9 "	0.000056 "	0.00024 "
Chloral	5.0 "	0.000071 "	0.00081 "
Barium chloride	6.5 "	0.000093 "	0.00008 "
Potassium chlorate	11.65 "	0.00017 "	0.0010 "
Lead acetate	28.3 "	0.00040 "	0.0081 "
Potassium nitrate	31.1 "	0.00044 "	0.0021 "
Ethyl alcohol	150. "	0.0021 "	0.008 "

After a consideration of the table and taking irto account the unreliability of the data on the toxic dose for man, it may be said that a certain relationship exists between the toxic dose for man and for the white mouse. Vanderkleed[10] suggests that the minimum lethal dose of a drug for animals of different species may be inversely proportional to the area of their surfaces. Some such relationship may exist between the white mouse and man, and the difference in toxic doses may be accounted for in this way. It will require more work to settle this point. For the present, it

is evident that we can not say that the fatal dose per gram for the mouse is the same as the fatal dose per gram for man. We hope that it will be possible to find a certain ratio existing between the minimum lethal dose for man and for the white mouse. Then a table of toxic coefficients using this test animal would be very valuable to toxicology and medico-legal cases. The white mouse appears to have an increased resistivity over that of man toward morphine and codeine. A number of peculiarities in reaction such as these would decrease the value of this research, since it was desired to find an animal which reacts to drugs in the same way that man does.

V. SUMMARY

1. The white mouse is a suitable animal for determining the toxic coefficients of drugs and other chemical substances for several reasons:

(a) Results accurate to within less than 10% can be obtained.

(b) The method has a wide applicability.

(c) Animals are cheap.

(d) Their food is inexpensive.

(e) They require but little attention.

(f) The technique is very simple.

(g) No expensive apparatus is necessary.

(h) In only a few cases are idiosyncrasies found.

(i) The resistivity of the white mouse is influenced by factors which may be controlled.

2. A substance should be adopted as a standard and other substances standardized on it as a base.

Caffeine is a suitable standard, for

1. Its injection gives sharp definite reactions in white mice.
2. Its action is fairly well understood.
3. It causes death chiefly by its action on the nervous system and the heart.
4. Its toxicity is sufficiently low to allow accurate measurement of doses.
5. No idiosyncrasies toward it were found.
6. The public will respect a table which uses it as a standard.

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ACKNOWLEDGMENT.

This study has been carried on under the auspices of the Pharmaceutical Research Fund. The writer wishes to express his thanks to the donors of the fund for the aid which they have given him by the establishment of a fellowship, and by furnishing an abundant supply of material.

The writer further wishes to express his thanks to the Director of the Fund, Dr. James H. Beal, who suggested the problems and aided him with many valuable suggestions, and to Mr. C. G. MacArthur for his continual aid in the progress of the work.

Urbana, Illinois, June 1915.

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